Embryonic induction: **Is the Nieuwkoop centre a useful concept?**

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Regionalisation of the amphibian embryo is classically thought to involve induction by the Spemann organiser, itself induced by the Nieuwkoop centre. This model has now been extended to teleosts, with the identification of a gene that appears to define the zebrafish equivalent of the Nieuwkoop centre.

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The vertebrate organiser, defined by Hans Spemann and Hilde Mangold 75 years ago, has received special attention from molecular embryologists since the early 1990s. This dorsal region of the gastrula embryo has been shown to pattern the axial mesoderm, endoderm and neural tissue in amphibians, zebrafish, chick and mouse [1]. A classical model proposes that, in the amphibian *Xenopus laevis*, formation of the Spemann organiser involves two sequential steps. The first is a process called cortical rotation, which takes place after fertilisation and triggers the relocalisation of dorsal determinants toward the future dorsal side of the embryo, thereby creating a first organising centre, the Nieuwkoop centre, in the dorsal vegetal cells (Figure 1a) [2]. The Nieuwkoop centre then emits signals that induce the Spemann organiser in the overlying equatorial region of the embryo, known as the dorsal blastopore lip.

At the molecular level, the only known consequence of the early cortical rotation in *Xenopus* embryos is the stabilisation of the signalling protein β-catenin and its translocation into dorsal nuclei. This, in turn, leads to the activation, in the dorsal vegetal cells of blastula stage embryos, of the early zygotic gene *Siamois*, which encodes a homeobox protein (Figure 1a) [2]. The model then proposes that *Siamois* plays a major role in the Nieuwkoop centre by activating, in overlying equatorial cells, the expression of genes for later organiser components, such as *goosecoid*, *chordin* and *Xlim-1*.

In teleost fishes, the embryo proper develops from the blastoderm which covers a unique yolk cell. At the mid-blastula stage, deep marginal blastoderm cells collapse and release their nuclei into the yolk cell, thus forming the yolk syncytial layer (YSL) [3]. Transplantation experiments have revealed that the yolk cell, after the 'mid-blastula transition' (when there are important changes, for example in the lengths of cell cycles), can induce mesoderm and organiser gene expression in the blastoderm [4]. The zebrafish equivalent of the frog Spemann organiser is called the shield, and forms in the dorsal blastoderm overlying the dorsal YSL [3]. As in frogs, formation of the organiser in early zebrafish embryos is dependent on the integrity of cortical arrays of microtubules, which presumably enable the transport of vegetally localised dorsal determinants [5], leading to the nuclear accumulation of β-catenin in the dorsal YSL and the dorsal blastoderm (Figure 1b) [6]. This, and the position of the dorsal YSL just underneath the shield, suggests that the dorsal YSL may be the source of the signals responsible for the induction of the shield, and thus be functionally equivalent to the frog Nieuwkoop centre [3].

Two recent papers [7,8] have now strengthened the link between the dorsal YSL and axial development in the zebrafish. These studies have identified a novel homeobox gene, named *dharma* [7] or *nieuwkoid* [8], as an important player in the acquisition of signalling properties by the dorsal YSL. Yamanaka *et al.* [7] isolated *dharma/nieuwkoid* through the first successful example of expression cloning in zebrafish, looking for genes with a dorsalising potential, whereas Koos and Ho [8] cloned *dharma/nieuwkoid* by looking for *paired*-like homeobox genes expressed at the gastrula stage. Alignment of the homeodomain of the encoded protein with those of other homeodomain proteins has failed to detect any orthologues of *dharma/nieuwkoid* in other vertebrates.

The *dharma/nieuwkoid* gene is expressed soon after the mid-blastula-transition, unilaterally on the side of the embryo where β-catenin shows nuclear localisation (see Figure 1b) [8]. This qualifies *dharma/nieuwkoid* as the earliest dorsal-specific gene known to date in zebrafish. It is initially expressed in the blastoderm [7,8], and 40 minutes later its expression can be detected in both blastoderm and dorsal YSL [8]. Another 30 minutes later, and until the onset of gastrulation, its expression becomes restricted to the dorsal YSL, suggesting that it might contribute to a Nieuwkoop-centre-like activity [7,8]. Consistently, *dharma/nieuwkoid* is expressed before the organiser gene *goosecoid* [7,8], and indeed it can induce *goosecoid* expression in a non-cell-autonomous manner when overexpressed in the blastoderm or in the yolk cell [7,8]. Taken together, these data suggest that *dharma/nieuwkoid* acts within the YSL to induce the organiser in the overlying

mesoderm cells. It is tempting to suggest that, in spite of sequence differences, *dharma/nieuwkoid* may play a similar role in the fish as *Siamois* does in the frog.

An important issue is whether *dharma/nieuwkoid* expression is a direct consequence of the nuclear accumulation of β-catenin in the dorsal YSL. Yamanaka *et al.* [7] report that the promoter region of *dharma/nieuwkoid* contains several consensus binding sites for Lef/Tcf, the cofactor that works with β-catenin to activate transcription of certain target genes in the nucleus, though no direct evidence that *dharma/nieuwkoid* really is regulated in this way has been reported yet. Furthermore, *dharma/nieuwkoid* expression is enhanced by lithium chloride treatment, which is known to trigger β-catenin signalling [7]. These observations suggest that there might be a direct connection between β-catenin and *dharma/nieuwkoid*.

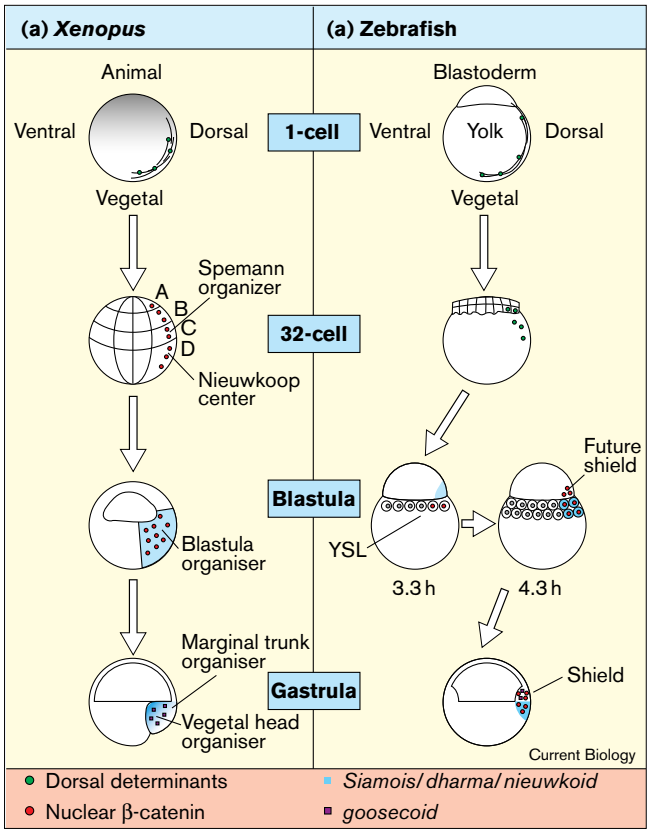
These two studies [7,8] strongly suggest that *dharma/nieuwkoid* is an important early regulator of axis formation in zebrafish, and unambiguously demonstrate that the yolk cell and YSL have organiser-inducing potential. They do not, however, provide a definitive demonstration that the dorsal YSL has a role in organiser induction during normal embryogenesis. We shall now address this issue by discussing a number of open questions. First, is *dharma/nieuwkoid* a major transducer of the β-catenin dorsal cue? Second, is *dharma/nieuwkoid* really a functional homologue of *Siamois*? And third, do the data gathered so far in *Xenopus* and zebrafish actually support a role for the Nieuwkoop centre in inducing the dorsal organiser?

A major transducer of β-catenin?

During early cleavages in zebrafish, the blastomeres and the yolk cell are interconnected by cytoplasmic bridges that allow the diffusion and homogeneous distribution of injected substances. Because of this, injection of *dharma/nieuwkoid* mRNA at the two-cell stage, in the blastoderm or in the yolk cell, provokes a broad ectopic expression of organiser genes such as *goosecoid*. This is later translated into a dramatic expansion of dorso-anterior axial structures, as revealed by morphological examination [7,8]. When two distant blastomeres are injected with *dharma/nieuwkoid* mRNA at the 16-cell stage, a discrete secondary axis forms, which can be visualised by the expression of the notochord marker *Sonic hedgehog* [8].

The *dharma/nieuwkoid* mRNA therefore behaves similarly to β-catenin, which can also induce the formation of a secondary body axis in fish embryos [9]. There are differences, however, as β-catenin can induce a complete body axis, although at low frequencies, whereas such an activity has not been observed for *dharma/nieuwkoid*. Although such differences need to be more carefully assessed in parallel experiments, they suggest that *dharma/nieuwkoid* does not mediate the full spectrum of β-catenin activities.

Figure 1



A model for the establishment of the dorsal organiser and axis formation in *Xenopus* and zebrafish embryos. **(a)** In *Xenopus*, cortical rotation moves dorsal determinants towards the future dorsal side of the embryo, creating a large domain where, starting at the 32-cell stage, β-catenin undergoes nuclear translocation. In the classical two-step model for organiser formation, the Nieuwkoop centre derives from dorsal-vegetal blastomeres (D), and the Spemann organiser forms in the progeny of dorsal marginal blastomeres (C). In an alternative model, at the mid-blastula stage, the domain where β-catenin is nuclear defines the blastula organiser and expresses *Siamois*, and at the gastrula stage, *Siamois* and other factors further define the vegetal head organiser and marginal trunk organiser. Note that *Siamois* and *goosecoid* are co-expressed. **(b)** In zebrafish, dorsal determinants are transported towards the future dorsal side of the embryo and enter the blastoderm. At the mid-blastula transition, β-catenin is translocated into dorsal yolk syncytial layer (YSL) nuclei. At this stage *dharma/nieuwkoid* is expressed in the dorsal blastoderm. A few minutes later, β-catenin appears in dorsal blastoderm nuclei, while *dharma/nieuwkoid* expression fades out in the blastoderm and can now be detected in the dorsal YSL. At the shield stage, *dharma/nieuwkoid* and *goosecoid* are expressed in the YSL and blastoderm, respectively, with no apparent overlap. In both species, the dorsal organiser forms where β-catenin is translocated into the nuclei, but downstream molecular events may differ significantly.

A functional homologue of *Siamois*?

In its relatively weak axis-inducing activity, compared with β-catenin, *dharma/nieuwkoid* differs from the frog Nieuwkoop factor *Siamois*. Injection of very low amounts of *Siamois* RNA in *Xenopus* embryos induces a complete secondary axis, indistinguishable from a β-catenin-induced

axis [2]. Injection of *dharma/nieuwkoid* RNA into vegetal blastomeres of frog embryos leads to the formation of poor ectopic axes, by no mean comparable to *Siamois*-induced secondary axes [7]. Finally, injection of *Siamois* RNA into zebrafish embryos does not induce a secondary axis or hyperdorsalisation of the primary axis ([10] and M. Hibi, personal communication).

The results of these inter-specific overexpression experiments suggest that the transcriptional targets of β-catenin may not be completely conserved between *Xenopus* and zebrafish. In this scheme, *dharma/nieuwkoid* would play in zebrafish a similar role to *Siamois* in frog, but through the activation of different targets, as suggested by sequence divergence in their respective homeodomains. This may explain the fact that, despite major efforts by several groups, no close homologue of *Siamois* has been identified, to date, in zebrafish or any other vertebrate.

Does the Nieuwkoop centre induce the dorsal organiser?

The concept of the Nieuwkoop centre originates from transplantation experiments in frogs (reviewed in [2]), which revealed the existence of dorsalising signals emanating from the progeny of dorsal vegetal blastomeres of 32-cell stage embryos (see Figure 1a). Although these experiments demonstrated that, like the dorsal YSL in fish, the frog dorsal vegetal blastomeres have dorsalising potential, it appears that this activity is not required for axial development.

Removal of the two dorsal-vegetal blastomeres (tier D in Figure 1a) at the 32-cell stage does not significantly affect axis formation in *Xenopus*, although it does impair normal gut development [11]. Furthermore, the combined removal of the two dorsal-marginal blastomeres of tier C and the two dorsal-animal blastomeres of tier B does not prevent axis formation either [11], indicating that the dorsal blastomeres may all have a common dorsalising potential and can act redundantly. This may relate to the observation that, at the 32-cell stage, β-catenin is detected in nuclei within a large dorsal region, including vegetal, marginal and animal blastomeres (Figure 1a) [2]. Similarly, in zebrafish, β-catenin is found to be nuclear in the dorsal blastoderm as well as in the dorsal YSL (Figure 1b) [6].

Support for the notion of a Nieuwkoop centre came from the apparent restricted localisation, as revealed by *in situ* hybridisation, of *Siamois* and *dharma/nieuwkoid* RNA in *Xenopus* vegetal cells and the zebrafish YSL, respectively. *Siamois* expression can, however, also be detected biochemically in the progeny of dorsal-marginal and dorsal-animal blastomeres isolated at the 32-cell stage, suggesting that it is also expressed in the Spemann organiser [12]. Similarly, *dharma/nieuwkoid* is first detected in the blastoderm, and one cannot rule out a possible function of this gene in the marginal zone as well. *Siamois* and *dharma/nieuwkoid* may therefore act both in the organiser and in the cells underlying this structure.

The broad distribution of β-catenin in dorsal nuclei suggests that axial development arises predominantly in an autonomous manner. Consistent with this, *goosecoid* expression can be detected in dissociated *Xenopus* dorsal blastomeres, demonstrating that intercellular signalling is dispensable for the establishment of an organiser genetic program [13]. Furthermore, *Siamois* and *goosecoid* are largely co-expressed in early gastrula (P. Lemaire, unpublished data; see Figure 1a), and Siamois protein can bind the promoter region of *goosecoid* to activate its transcription [2]. The situation is different for *dharma/nieuwkoid*, as it does not seem to be co-expressed with *goosecoid* in the blastoderm [7,8] and its overexpression cannot induce *goosecoid* expression in the YSL [7,8]. The *dharma/nieuwkoid* gene appears to act non-autonomously to induce *goosecoid*, contrasting sharply with the fact that β-catenin induces *goosecoid* expression in an autonomous manner in zebrafish [9]. This suggests that β-catenin can induce the organiser independently of *dharma/nieuwkoid*, by its direct action in marginal cells.

If the Nieuwkoop centre is dispensable for axial development, how then is the organiser specified? An alternative model could be proposed, in which the translocation of β-catenin into dorsal nuclei leads to the activation of early zygotic regulators, such as *Siamois* or *dharma/nieuwkoid*, in a large dorsal domain, giving rise to the blastula organiser [14]. The dorsal information provided by these early blastula organiser factors is then interpreted differently by vegetal and marginal cells to give rise to at least two distinct domains of the gastrula organiser: a dorsal-vegetal organiser involved in head formation and fated to form the dorso-anterior endoderm, and a dorsal-marginal organiser required for trunk formation (Figure 1a).

Such a model is supported by the fact that, in *Xenopus*, *Siamois* can differentially induce the head-specific secreted molecule Cerberus in vegetal cells and the trunk-specific secreted factor Chordin in animal cells [15]. In this model, one could reason that the Nieuwkoop centre is in fact required for regionalisation of the endoderm in the same way the Spemann organiser is necessary for regionalisation of the mesoderm. This possibility can now be addressed in the frog, where an increasing number of endoderm regional markers are available.

In conclusion, the isolation of *dharma/nieuwkoid* is an important step forward in understanding organiser formation in zebrafish, and gives us a handle to look for upstream and downstream effectors in this process. The studies reviewed here also provide good evidence for the idea that the molecular events responsible for organiser formation may diverge downstream of β-catenin between

fish and frogs, with *dharma/nieuwkoid* taking over some of the functions of *Siamois*. The zebrafish, as a genetically tractable model, will undoubtedly facilitate elucidation of the molecular processes involved in organiser formation and axis development. As *dharma/nieuwkoid* is a true factor of the blastula organiser, it will be very interesting to determine whether it is required for the expression of organiser genes, such as *goosecoid*, and if so in which tissue it acts. By making reciprocal recombinations of mutant and wild-type blastoderm and yolk cells, it should be possible to address this important issue directly.

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References

1. Tam PPL, Behringer, RR: **Mouse gastrulation: the formation of a mammalian body plan.** *Mech Dev* 1997, **68:**3-25.
2. Moon RT, Kimelman D: **From cortical rotation to organizer gene expression: toward a molecular explanation of axis specification in *Xenopus*.** *Bioessays* 1998, **20:**536-545.
3. Schier AF, Tabot WS: **The zebrafish organizer.** *Curr Opin Genet Dev* 1998, **8:**464-471.
4. Mizuno T, Yamaha M, Wakahara A, Kuroiwa A, Takeda H: **Mesoderm induction in zebrafish.** *Nature* 1996, **383:**131-132.
5. Jesuthasan S, Strähle U: **Dynamic microtubules and specification of the zebrafish embryonic axis.** *Curr Biol* 1996, **7:**31-42.
6. Schneider S, Steinbeisser H, Warga R, Hausen P: **β-catenin translocation into nuclei demarcates the dorsalizing center in frog and fish embryos.** *Mech Dev* 1996, **57:**191-198.
7. Yamanaka Y, Mizuno T, Sasai Y, Kishi M, Takeda H, Kim C-H, Hibi M, Hirano T: **A novel homeobox gene, *dharma*, can induce the organizer in a non-cell-autonomous manner.** *Genes Dev* 1998, **12:**2345-2353.
8. Koos DS, Ho R: **The *nieuwkoid* gene characterizes a Nieuwkoop center-like activity in the zebrafish.** *Curr Biol* 1998, **8:**1199-1206.
9. Kelly GM, Erezyilmaz DF, Moon RT: **Induction of a secondary body axis in zebrafish occurs following the overexpression of β-catenin.** *Mech Dev* 1995, **53:**261-273.
10. Sumoy L, Kiefer J, Kimelman D: **Conservation of intracellular Wnt signalling components in dorso-ventral axis formation in zebrafish.** *Dev Genes Evol*, in press.
11. Kageura H: **Three regions of the 32-cell embryo of *Xenopus laevis* essential for formation of a complete tadpole.** *Dev Biol* 1995, **170:**376-386.
12. Ding X, Hausen P, Steinbeisser H: **Pre-mid-blastula-transition patterning of early gene regulation in *Xenopus*: the role of the cortical rotation and mesoderm induction.** *Mech Dev* 1998, **70:**15-24.
13. Lemaire P, Gurdon JB: **A role for the cytoplasmic determinants in mesoderm patterning: cell-autonomous activation of the *goosecoid* and *XWnt-8* genes along the dorso-ventral axis of early Xenopus embryos.** *Development* 1994, **120:**1191-1199.
14. Gerhart J, Doniach T, Stewart R: **Organizing the *Xenopus* organizer.** In *Gastrulation, Movements, Patterns and Molecules.* Edited by Keller R, Clark WH, Griffin F. New York: Plenum Press; 1991:57-78.
15. Darras S, Marikawa Y, Elinson RP, Lemaire P: **Animal and vegetal pole cells of early *Xenopus* embryos respond differently to maternal dorsal determinants: implications for the patterning of the organiser.** *Development* 1997, **124:**4275-4286.